

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

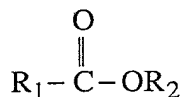
1. A method of enhancing the transient expression of a foreign gene in a eukaryotic cell in vivo comprising:

introducing into the cell a molecule of foreign DNA that encodes a protein in a form capable of being expressed in the cell; and

contacting the cell before, during, or after introducing the DNA with a biocompatible transient expression enhancing agent, provided that said agent is not butyrate or butyric acid.

2. The method of Claim 1 further comprising the step of detecting the foreign protein in the cell after contacting the cell with a biocompatible transient expression enhancing agent.

3. The method of Claim 1, wherein the transient expression enhancing agent comprises at least one carboxylic acid derivative having the formula:



wherein R<sub>1</sub> is:

CHNH<sub>2</sub>R<sub>3</sub>, wherein R<sub>3</sub> is the side chain of a naturally occurring amino acid;

C<sub>6</sub>H<sub>4</sub>R<sub>4</sub>, wherein R<sub>4</sub> is H, CH<sub>3</sub>, (CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>, NH<sub>2</sub>, COCH<sub>3</sub>, CO(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>, C(CH<sub>3</sub>)<sub>3</sub>, CH(CH<sub>3</sub>)<sub>2</sub>, (CH<sub>2</sub>)<sub>n</sub>CH(CH<sub>3</sub>)<sub>2</sub>, (CH<sub>2</sub>)<sub>n</sub>COCH<sub>3</sub>, OCH<sub>3</sub>, or O(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>, wherein n = 1-3;

CHNH<sub>2</sub>(CH<sub>2</sub>)<sub>n</sub>R<sub>5</sub>, wherein n = 1-7 and R<sub>5</sub> is CH<sub>3</sub>, OH, CONH<sub>2</sub>, C<sub>6</sub>H<sub>4</sub>OH, or CONHNH<sub>2</sub>;

(CH<sub>2</sub>)<sub>n</sub>R<sub>6</sub>, wherein n = 1-9 and R<sub>6</sub> is an indole group, NCH<sub>3</sub>C(=NH)NH<sub>2</sub>, SCH<sub>3</sub>, NH<sub>2</sub>, CH<sub>3</sub>, CO<sub>2</sub>H, CONH<sub>2</sub>, or NHC(=NH)NH<sub>2</sub>, provided that when n=2 and R<sub>2</sub> is H or M, R<sub>6</sub> is not CH<sub>3</sub>;

(CH<sub>2</sub>)<sub>n</sub>CHNH<sub>2</sub>CO<sub>2</sub>H, wherein n = 1-8;

CH(CO<sub>2</sub>H)NHCONH<sub>2</sub>; or,

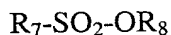
C<sub>5</sub>H<sub>4</sub>N; and

wherein  $R_2$  is selected from  $H$ ,  $CH_3$ ,  $(CH_2)_nCH_3$  wherein  $n = 1-8$ ,  $(CH_2)_xO(CH_2)_yCH_3$  or  $(CH_2)_xCO(CH_2)_yCH_3$  wherein  $x + y = 2-7$ , or  $M$ , wherein  $M$  is a metal counterion or a low molecular weight organic cation.

4. The method of Claim 3 wherein the transient expression enhancing agent comprises an amino acid derivative selected from the group consisting of 3-methyl-L-histidine,  $\alpha$ -ketoglutaric acid,  $\beta$ -alanine, carnosine, citrulline, creatine, folic acid, glutathione, hippuric acid, homoserine, N-(4-aminobenzyl)-L-glutamic diethylester, N-carbamyl aspartic acid, N-formyl-L-methionine, and ornithine.

5. The method of Claim 3, wherein  $R_1$  is non-polar or hydrophobic at a pH between 4.5 and 10.5.

6. The method of Claim 1, wherein the transient expression enhancing agent comprises a sulfonic acid derivative having the formula:



wherein  $R_7$  is a lower alkyl, aryl, substituted lower alkyl, aryl, substituted lower alkyl, or substituted aryl; and

$R_8$  is a hydrogen, a metal counterion, or a low molecular weight organic cation.

7. The method of Claim 6 wherein  $R_7$  is an amino substituted lower alkyl group or an amino substituted aryl group.

8. The method of Claim 6 wherein the sulfonic acid derivative is selected from the group consisting of 3-aminobenzene sulfonic acid, taurine, and salts thereof.

9. The method of Claim 1, wherein the transient expression enhancing agent comprises a glycosaminoglycan.

10. The method of Claim 9, wherein the glycosaminoglycan is a sulfonated amino polysaccharide.

11. The method of Claim 10, wherein the sulfonated amino polysaccharide comprises an N-acetylated amino polysaccharide.

12. The method of Claim 11, wherein the N-acetylated amino polysaccharide is chondroitin sulfate.

13. The method of Claim 1 wherein the transient expression enhancing agent comprises a compound selected from the group consisting of adrenaline, coenzyme B12, and methylcobalamin.

14. The method of Claim 1, wherein the agent comprises: benzoic acid and 4-ethylbenzoic acid; or benzoate buffer and chondroitin sulfate; or benzoate buffer and glutamic acid; or glutathione, methionine, glycine,  $\alpha$ -amino-n-butyric acid, taurine, phenylalanine, benzoate buffer, and alanine; or 4-ethylbenzoic acid, benzoic acid, and chondroitin sulfate; or  $\alpha$ -lipoic acid and chondroitin sulfate.

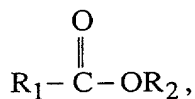
15. The method of Claim 1, wherein the concentration of the transient expression enhancing agent is 1-15 mM.

16. The method of Claim 10, wherein the concentration of the transient expression enhancing agent is 0.01-0.5 mM.

17. The method of Claim 12, wherein the chondroitin sulfate has an average molecular mass of no greater than 9000 daltons.

18. The method of Claim 12, wherein the chondroitin sulfate has an average molecular mass of no greater than 4000 daltons.

19. The method of Claim 1, wherein the cell is contacted with a first transient expression enhancing agent prior to and during the introduction into the cell of the foreign DNA, wherein the agent comprises at least one compound having the formula:



wherein  $\text{R}_1$  is:

$\text{CHNH}_2\text{R}_3$ , wherein  $\text{R}_3$  is the side chain of a naturally occurring amino acid;

$C_6H_4R_4$ , wherein  $R_4$  is H,  $CH_3$ ,  $(CH_2)_nCH_3$ ,  $NH_2$ ,  $COCH_3$ ,  $CO(CH_2)_nCH_3$ ,  $C(CH_3)_3$ ,  $CH(CH_3)_2$ ,  $(CH_2)_nCH(CH_3)_2$ ,  $(CH_2)_nCOCH_3$ ,  $OCH_3$ , or  $O(CH_2)_nCH_3$ , wherein  $n = 1-3$ ;

$CHNH_2(CH_2)_nR_5$ , wherein  $n = 1-7$  and  $R_5$  is  $CH_3$ ,  $OH$ ,  $CONH_2$ ,  $C_6H_4OH$ , or  $CONHNH_2$ ;

$(CH_2)_nR_6$ , wherein  $n = 1-9$  and  $R_6$  is an indole group,  $NCH_3C(=NH)NH_2$ ,  $SCH_3$ ,  $NH_2$ ,  $CH_3$ ,  $CO_2H$ ,  $CONH_2$ , or  $NHC(=NH)NH_2$ , provided that when  $n=2$  and  $R_2$  is H or M,  $R_6$  is not  $CH_3$ ;

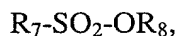
$(CH_2)_nCHNH_2CO_2H$ , wherein  $n = 1-8$ ;

$CH(CO_2H)NHCONH_2$ ; or

$C_5H_4N$ ; and

wherein  $R_2$  is H,  $CH_3$ ,  $(CH_2)_nCH_3$  wherein  $n = 1-8$ , or  $(CH_2)_xO(CH_2)_yCH_3$  or  $(CH_2)_xCO(CH_2)_yCH_3$  wherein  $x + y = 2-7$ , or M, wherein M is a metal counterion or a low molecular weight organic cation;

or the first transient expression enhancing agent comprises a compound having the formula:



wherein  $R_7$  is a lower alkyl, aryl, substituted lower alkyl, or substituted aryl; and

$R_8$  is a hydrogen, a metal counterion, or a low molecular weight organic cation;

and following the introduction of the foreign DNA, the cell is contacted with a second transient expression enhancing agent, wherein the second agent comprises a sulfonated amino polysaccharide.

20. The method of Claim 19, wherein the cell further is continuously exposed to the first agent after the introduction of the foreign DNA into the cell.

21. The method of Claim 1, wherein the cell is present in a live host, and the transient expression enhancing agent is introduced into the host orally or by injection.

22. The method of Claim 1, wherein the foreign DNA is introduced into the cell by a method selected from the group consisting of lipofection, a viral vector,

exposure of cells to coprecipitates of calcium phosphate, and transfection in the presence of a dendrimer.

23. The method of Claim 22, wherein the DNA is introduced into the cell by a viral vector, and the viral vector comprises an adenovirus.

24. The method of Claim 1, wherein the agent contains at least one acidic moiety and at least one moiety that is hydrophobic at a pH between 4.5 and 10.5, and wherein the acidic group may be modified to form a salt or an ester.

25. The method of Claim 24, wherein the acidic moiety is hydrophobic and organic at a pH between 4.5 and 10.5.

26. A method of screening an agent comprising at least one chemical compound to determine whether the agent is capable of enhancing the transient expression of a foreign gene in a eukaryotic cell, wherein the agent is biocompatible and contains at least one hydrophobic moiety and at least one acidic moiety, the method comprising the steps of:

introducing into a first and a second SW480 P3 cell on day 0 a molecule of foreign DNA that encodes a protein in a form capable of being expressed in the cells;

before, during, or after introducing the DNA, contacting the second cell with the agent;

cumulatively measuring in both cells between days 0 and 4, or between days 4 and 7, or between days 4 and 14 the amount of protein expressed from the foreign DNA, and using these amounts to determine, respectively, a value for  $X$ ,  $G_7$ , or  $G_{14}$  according to the formula:

$$X, \text{ or } G_7 \text{ or } G_{14} = 100 - \frac{(A \times 100)}{C},$$

wherein "A" is the amount of the protein encoded by the foreign gene expressed in the first cell and, "C" is the amount of protein expressed in the second cell; and,

determining that the agent is capable of enhancing transient expression if  $X$  or  $G_7$  or  $G_{14}$  is greater than 10.

27. The method of Claim 26, wherein  $X$  or  $G_7$  or  $G_{14}$  is greater than 25.

28. A method of enhancing the transient expression of a foreign gene in a cell comprising:

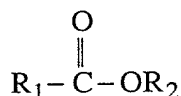
introducing into the cell a molecule of foreign DNA that encodes a protein in a form capable of being expressed in the cell; and,

contacting the cell with an agent for which  $X$  or  $G_7$  or  $G_{14}$  is greater than 25 when the agent is evaluated according to the assay of Claim 26.

29. A method of manipulating the metabolism of a cell to reduce the cell's consumption of glucose, comprising the step of contacting the cell with an agent that induces the cell to use proteins or amino acids as their primary energy source.

30. The method of Claim 29, wherein the agent further induces the cell to express an endogenous phosphatase enzyme activity.

31. The method of Claim 29, wherein said agent is capable of enhancing the transient expression of a foreign gene in the cell, wherein the agent comprises at least one chemical compound having the formula:



wherein  $\text{R}_1$  is:

$\text{CHNH}_2\text{R}_3$ , wherein  $\text{R}_3$  is the side chain of a naturally occurring amino acid;

$\text{C}_6\text{H}_4\text{R}_4$ , wherein  $\text{R}_4$  is  $\text{H}$ ,  $\text{CH}_3$ ,  $(\text{CH}_2)_n\text{CH}_3$ ,  $\text{NH}_2$ ,  $\text{COCH}_3$ ,  $\text{CO}(\text{CH}_2)_n\text{CH}_3$ ,  $\text{C}(\text{CH}_3)_3$ ,  $\text{CH}(\text{CH}_3)_2$ ,  $(\text{CH}_2)_n\text{CH}(\text{CH}_3)_2$ ,  $(\text{CH}_2)_n\text{COCH}_3$ ,  $\text{OCH}_3$ , or  $\text{O}(\text{CH}_2)_n\text{CH}_3$ , wherein  $n = 1-3$ ;

$\text{CHNH}_2(\text{CH}_2)_n\text{R}_5$ , wherein  $n = 1-7$  and  $\text{R}_5$  is  $\text{CH}_3$ ,  $\text{OH}$ ,  $\text{CONH}_2$ ,  $\text{C}_6\text{H}_4\text{OH}$ , or  $\text{CONHNH}_2$ ;

$(\text{CH}_2)_n\text{R}_6$ , wherein  $n = 1-9$  and  $\text{R}_6$  is an indole group,  $\text{NCH}_3\text{C}(=\text{NH})\text{NH}_2$ ,  $\text{SCH}_3$ ,  $\text{NH}_2$ ,  $\text{CH}_3$ ,  $\text{CO}_2\text{H}$ ,  $\text{CONH}_2$ , or  $\text{NHC}(=\text{NH})\text{NH}_2$ , provided that when  $n=2$  and  $\text{R}_2$  is  $\text{H}$  or  $\text{M}$ ,  $\text{R}_6$  is not  $\text{CH}_3$ ;

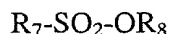
$(\text{CH}_2)_n\text{CHNH}_2\text{CO}_2\text{H}$ , wherein  $n = 1-8$ ;

$\text{CH}(\text{CO}_2\text{H})\text{NHCONH}_2$ ; or,

$\text{C}_5\text{H}_4\text{N}$ ; and

wherein  $R_2$  is H,  $CH_3$ ,  $(CH_2)_nCH_3$  wherein  $n = 1-8$ ,  $(CH_2)_xO(CH_2)_yCH_3$  or  $(CH_2)_xCO(CH_2)_yCH_3$  wherein  $x + y = 2-7$ , or M, wherein M is a metal counterion or a low molecular weight organic cation; or

the group consisting of a sulfonic acid derivative having the formula:



wherein  $R_7$  is a lower alkyl, aryl, substituted lower alkyl, or substituted lower aryl; and

$R_8$  is a hydrogen atom, a metal counterion, or a low molecular weight organic cation; or

a sulfonated amino polysaccharide.

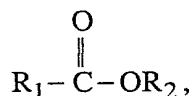
32. The method of Claim 31, wherein the agent comprises a chemical compound selected from the group consisting of benzoic acid, 4-ethylbenzoic acid, benzoate buffer, and chondroitin sulfate.

33. The method of Claim 31, wherein the agent is administered *in vivo* to a mammal.

34. A method of enhancing the transient expression of a foreign gene in a eukaryotic cell *in vivo* comprising:

introducing into the cell a molecule of foreign DNA that encodes a protein in a form capable of being expressed in the cell;

contacting the cell with a first agent during the introduction into the cell of the foreign DNA, wherein the first agent comprises at least one chemical compound having the formula:



wherein  $R_1$  is:

$CHNH_2R_3$ , wherein  $R_3$  is the side chain of a naturally occurring amino acid;

$C_6H_4R_4$ , wherein  $R_4$  is H,  $CH_3$ ,  $(CH_2)_nCH_3$ ,  $NH_2$ ,  $COCH_3$ ,  $CO(CH_2)_nCH_3$ ,  $C(CH_3)_3$ ,  $CH(CH_3)_2$ ,  $(CH_2)_nCH(CH_3)_2$ ,  $(CH_2)_nCOCH_3$ ,  $OCH_3$ , or  $O(CH_2)_nCH_3$ , wherein  $n = 1-3$ ;

$\text{CHNH}_2(\text{CH}_2)_n\text{R}_5$ , wherein  $n = 1-7$  and  $\text{R}_5$  is  $\text{CH}_3$ ,  $\text{OH}$ ,  $\text{CONH}_2$ ,  $\text{C}_6\text{H}_4\text{OH}$ , or  $\text{CONHNH}_2$ ;

$(\text{CH}_2)_n\text{R}_6$ , wherein  $n = 1-9$  and  $\text{R}_6$  is an indole group,  $\text{NCH}_3\text{C}(=\text{NH})\text{NH}_2$ ,  $\text{SCH}_3$ ,  $\text{NH}_2$ ,  $\text{CH}_3$ ,  $\text{CO}_2\text{H}$ ,  $\text{CONH}_2$ , or  $\text{NHC}(=\text{NH})\text{NH}_2$ , provided that when  $n=2$  and  $\text{R}_2$  is  $\text{H}$  or  $\text{M}$ ,  $\text{R}_6$  is not  $\text{CH}_3$ ;

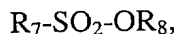
$(\text{CH}_2)_n\text{CHNH}_2\text{CO}_2\text{H}$ , wherein  $n = 1-8$ ;

$\text{CH}(\text{CO}_2\text{H})\text{NHCONH}_2$ ; or,

$\text{C}_5\text{H}_4\text{N}$ ; and

wherein  $\text{R}_2$  is  $\text{H}$ ,  $\text{CH}_3$ ,  $(\text{CH}_2)_n\text{CH}_3$  wherein  $n = 1-8$ ,  $(\text{CH}_2)_x\text{O}(\text{CH}_2)_y\text{CH}_3$  or  $(\text{CH}_2)_x\text{CO}(\text{CH}_2)_y\text{CH}_3$  wherein  $x + y = 2-7$ , or  $\text{M}$ , wherein  $\text{M}$  is a metal counterion or a low molecular weight organic cation;

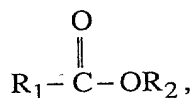
or the first agent comprises at least one chemical compound having the formula:



wherein  $\text{R}_7$  is a lower alkyl, aryl, substituted lower alkyl, or substituted lower aryl; and

$\text{R}_8$  is a hydrogen atom, a metal counterion, or a low molecular weight organic cation;

and during and/or following the introduction of the foreign DNA, the cell is contacted with a second agent, wherein the second agent comprises at least one sulfonated amino polysaccharide or wherein the second agent comprises at least one chemical compound having the formula:



wherein  $\text{R}_1$  is:

$\text{CHNH}_2\text{R}_3$ , wherein  $\text{R}_3$  is the side chain of a naturally occurring amino acid;

$\text{C}_6\text{H}_4\text{R}_4$ , wherein  $\text{R}_4$  is  $\text{H}$ ,  $\text{CH}_3$ ,  $(\text{CH}_2)_n\text{CH}_3$ ,  $\text{NH}_2$ ,  $\text{COCH}_3$ ,  $\text{CO}(\text{CH}_2)_n\text{CH}_3$ ,  $\text{C}(\text{CH}_3)_3$ ,  $\text{CH}(\text{CH}_3)_2$ ,  $(\text{CH}_2)_n\text{CH}(\text{CH}_3)_2$ ,  $(\text{CH}_2)_n\text{COCH}_3$ ,  $\text{OCH}_3$ , or  $\text{O}(\text{CH}_2)_n\text{CH}_3$ , wherein  $n = 1-3$ ;

$\text{CHNH}_2(\text{CH}_2)_n\text{R}_5$ , wherein  $n = 1-7$  and  $\text{R}_5$  is  $\text{CH}_3$ ,  $\text{OH}$ ,  $\text{CONH}_2$ ,  $\text{C}_6\text{H}_4\text{OH}$ , or  $\text{CONHNH}_2$ ;



$(CH_2)_nR_6$ , wherein  $n = 1-9$  and  $R_6$  is an indole group,  $NCH_3C(=NH)NH_2$ ,  $SCH_3$ ,  $NH_2$ ,  $CH_3$ ,  $CO_2H$ ,  $CONH_2$ , or  $NHC(=NH)NH_2$ , provided that when  $n=2$  and  $R_2$  is H or M,  $R_6$  is not  $CH_3$ ;

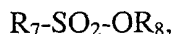
$(CH_2)_nCHNH_2CO_2H$ , wherein  $n = 1-8$ ;

$CH(CO_2H)NHCONH_2$ ; or,

$C_5H_4N$ ; and

wherein  $R_2$  is H,  $CH_3$   $(CH_2)_nCH_3$  wherein  $n = 1-8$   $(CH_2)_xO(CH_2)_yCH_3$  or  $(CH_2)_xCO(CH_2)_yCH_3$  wherein  $x + y = 2-7$ , or M, wherein M is a metal counterion or a low molecular weight organic cation;

or the second agent comprises at least one chemical compound having the formula:



wherein  $R_7$  is a lower alkyl, aryl, substituted lower alkyl, or substituted lower aryl; and

$R_8$  is a hydrogen atom, a metal counterion, or a low molecular weight organic cation; and

wherein the first agent is one that has a value for  $X$  that is greater than 25 when  $X$  is calculated according to the formula:

$$X = 100 - \frac{(A \times 100)}{C}$$

and wherein the second agent is one that has a value of  $G_7$  or  $G_{14}$  that is greater than 25, wherein  $G_7$  or  $G_{14}$  is calculated according to the formula:

$$G_7 \text{ or } G_{14} = 100 - \frac{(A \times 100)}{C}$$

wherein for both  $X$  or  $G_7$  or  $G_{14}$ , "A" is the amount of the protein encoded by the foreign gene expressed in a first cell that is contacted with the first and the second agent, and "C" is the amount of protein expressed in a second cell that is not contacted with the first or the second agent.

35. The method of Claim 34, wherein the cell is contacted with the first agent prior to, continuously after, or both prior to and continuously after the introduction of the foreign DNA.

36. The method of Claim 34, wherein the first agent comprises benzoate buffer, and the second agent comprises chondroitin sulfate.

37. The method of Claim 34, wherein the first agent comprises benzoic acid and 4-ethylbenzoic acid, and the second agent comprises benzoate buffer and chondroitin sulfate.

38. The method of Claim 34, wherein the first agent comprises benzoate buffer and glutamic acid, and the second agent comprises chondroitin sulfate.

39. The method of Claim 34, wherein the cell is contacted with the first agent for about 24 hours prior to the introduction into the cell of the foreign DNA.

40. A method of enhancing the transient expression of a foreign gene in a eukaryotic cell in vivo comprising:

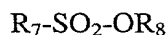
introducing into the cell a molecule of foreign DNA that encodes a protein in a form capable of being expressed in the cell; and,

contacting the cell before, during, or after introducing the DNA with a transient expression enhancing agent;

wherein the transient expression enhancing agent comprises:

a compound selected from the group consisting of 3-methyl-L-histidine,  $\alpha$ -ketoglutaric acid,  $\beta$ -alanine, carnosine, citrulline, creatine, glutathione, hippuric acid, homoserine, N-carbamyl aspartic acid, N-formyl-L-methionine, ornithine, N-(4-aminobenzyl)-L-glutamic diethylester, ethyl-4-acetylbutyrate, adrenaline, methylcobalamin, benzoic acid, benzoate buffer, 4-ethylbenzoic acid and a sulfonated N-acetylated amino polysaccharide, disaccharide monomeric units derived from Type C chondroitin sulfate, polymannose, mannose; or

a sulfonic acid derivative having the formula:



wherein  $R_7$  is a lower alkyl, aryl, substituted lower alkyl, aryl, substituted lower alkyl, or substituted aryl, and  $R_8$  is a hydrogen, a metal counterion, or a low molecular weight organic cation; or

benzoic acid and 4-ethylbenzoic acid; or

benzoic acid and 4-ethylbenzoic acid and chondroitin sulfate; or

benzoic acid and L-glutamine; or

benzoate buffer and chondroitin sulfate; or

benzoate buffer and glutamic acid; or

lipoic acid and chondroitin sulfate; or

benzoate buffer, chondroitin sulfate, and L-glutamine; or

chondroitin sulfate and L-glutamine; or

butyrate buffer and L-glutamine; or

a mixture comprising glutathione, methionine, glycine,  $\alpha$ -amino-n-butyric acid, taurine, phenylalanine, benzoate buffer, and alanine.